general implication, high abundances of elements such as Ir or Os may therefore be ambiguous as sole indicators of an impact origin or a cosmic component in impact-derived rocks. However, the $^{187}Os/^{188}Os$ ratios in all rocks from the Bosumtwi crater are high and typical for old continental crust, which supports the conclusion that the bulk of the Os in the tektites is of extraterrestrial, and not crustal, origin. The $^{187}Os/^{188}Os$ ratios in the Ivory Coast tektites are compatible with those in both chondritic and iron projectiles, but the Cr enrichment in the tektites seems to favor a projectile of chondritic composition. These results support the conclusion that no endogenic process can explain the origin of tektites and that the first, high-temperature ejecta formed during impact melting contain a small but significant projectile component.

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32. For Re and Os analyses, we used about 2 to 3 g of tektite, 2 g of impact glass, and 8 to 10 g of target rocks. Whole-rock powders were spiked with enriched ^{190}Os and ^{185}Re. Dissolution and spike equilibration were achieved by repeated acid digestion drying steps, followed by reflux distillation for Os [R. J. Walker, *Anal. Chem.* **60**, 1231 (1988)] and anion-exchange separation for Re [J. W. Morgan and R. J. Walker, *Anal. Chim. Acta* **222**, 291 (1989); J. W. Morgan, D. W. Golightly, J. A. F. Dorrzapf, *Talanta* **38**, 259 (1991)]. The laboratory procedures were optimized to yield low Re and Os blanks (Table 1).
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Greater Susceptibility to Mutations in Lagging Strand of DNA Replication in *Escherichia coli* Than in Leading Strand

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Models of DNA replication in *Escherichia coli* involve an asymmetric DNA polymerase complex that replicates concurrently the leading and the lagging strands of double-stranded DNA. The effect of asymmetry on mutagenesis was tested with pairs of plasmids containing the unidirectional ColE1 origin of replication and a single lesion located in the leading or lagging strand. The lesion used was the covalent adduct that the chemical carcinogen *N*-2-acetylaminofluorene (AAF) forms with the C-8 position of guanine. Whether SOS was induced or not, mutations arose at about a 20-fold higher frequency when the AAF adduct was located in the lagging strand than when in the leading strand.

Carcinogens such as the heterocyclic food mutagens and 4-aminobiphenyl are found bound to the C-8 position of guanine in DNA in human colon and pancreas tissue, where these chemicals are suspected to be involved in tumor formation (*1*). *N*-2-Acetylaminofluorene (AAF) is another of this family of aromatic compounds. We wished to investigate the mechanism by which these compounds induce mutations. In *E. coli*, AAF adducts induce frameshift mutations when located within two types of DNA sequences that are mutation hot spots (*2*, *3*): series of at least three guanines, in which AAF induces single nucleotide deletions (*4*), and the Nar I sequence (GGCGCC), in which AAF induces two-nucleotide deletions (*5*). These mutations may result from two distinct mutagenesis pathways, as suggested by their different genetic requirements (*6*).

Plasmid pUC8 is a ColE1-derived plasmid whose unidirectional replication relies entirely on host-encoded proteins (*7*, *8*). Plasmid pUC8 encodes both the *lacZ* α-complementing and the β-lactamase genes. We placed a single AAF adduct in the nontranscribed strand of the *lacZ* α-complementing gene. In the parent pUC8 plasmid this strand is the lagging strand for DNA replication (referred to as the lagging orientation). Reversing a restriction fragment containing the entire *lacZ* α-complementing gene places the adduct on the leading strand of replication without altering either its sequence context or its status with respect to transcription (referred to as the leading orientation) (Fig. 1A). We constructed plasmids in both lag-

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ging and leading orientations, containing single AAF adducts in each of the mutation hot spot sequences (Fig. 1). For the sequence 5′-$G^1G^2G^3$-3′, the AAF adduct was located at guanine G^3, which is the most mutagenic position (*4*). For the Nar I sequence 5′-$G^1G^2CG^3$CC-3′, the AAF adduct was located at guanine G^3, which is the only guanine in the sequence able to trigger double-nucleotide deletions (*5*). Covalently closed plasmids containing single adducts were introduced into a strain of *E. coli*, JM103, carrying the *uvrA6* mutant allele; these cells are deficient in excision repair. Thus, excision of the single adduct is prevented, and the mutation frequency is about 10-fold higher than in a repair-proficient strain (*4*). The mutation frequency was approximately 20-fold higher when the adduct was located in the lagging strand than when in the leading strand for both mutation hot spots (Table 1). The bias in mutation frequencies was similar whether or not SOS was induced (Table 1). Induction of the SOS functions by irradiation of the bacteria with ultraviolet light before transformation increased mutation frequencies similarly in both strands. However, the two hot spot sequences responded differently: Mutations at GGG increased 50-fold and mutations at GGCGCC increased 5-fold. This difference is not unexpected, in that the single and double nucleotide deletion events have different SOS requirements (*6, 9*). When the adduct is placed in the lagging strand, replication and *lacZ* transcription proceed in the same direction; when in the leading strand, replication and transcription proceed in opposite directions (Fig. 1A). However, repression or derepression of *lacZ* transcription had no effect on the observed mutation frequencies (*10*). Therefore, the asymmetry of mutagenesis is not due to an interaction between replication and transcription.

The observed difference in mutation frequency between the two strands may be due to the lagging strand polymerase complex being more mutagenic than the leading strand polymerase complex. The mechanism of single-nucleotide deletion involves incorporation of a cytosine residue opposite the modified guanine, then slippage such that the cytosine residue becomes base-paired with the guanine residue located 5′ to the adducted guanine in the template strand, which forms a slipped mutagenic intermediate (SMI) (*4*). The SMI is thought to be a transient intermediate in equilibrium with the original primer-template alignment. Elongation from the SMI would be mutagenic, whereas elongation from the original primer-template substrate would be error free. The formation of the SMI is thermodynamically favored by the presence of an AAF adduct (*11*). Although not formally established, the mechanism by which the double nucleotide deletions are formed may be similar.

We postulate that the lagging strand polymerase is capable of elongating from the SMI at about a 20-fold higher frequency than is the leading strand polymerase. Concurrent replication of the two antiparallel strands of DNA would require that the DNA polymerase III complex be asymmetric (*12, 13*) as supported by our observations. However, it should be stressed that ColE1 plasmids require polymerase I for initiation of leading strand synthesis and then switch rapidly to polymerase III (*14*). Although the Pol I–Pol III switch has not

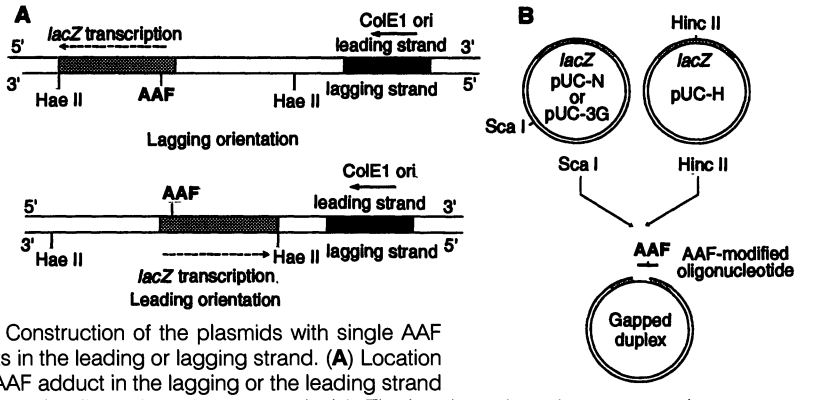

Fig. 1. Construction of the plasmids with single AAF adducts in the leading or lagging strand. (**A**) Location of the AAF adduct in the lagging or the leading strand (lagging or leading orientation, respectively). The lagging orientation presents the same organization as plasmid pUC8. The leading orientation was obtained by inversion of the Hae II restriction fragment of pUC8 that encompasses the entire *lac* region [pUC8(L)]. Plasmids designated pUC-3G(I) and pUC-3G(L) represent the constructions where AAF is located on the third G residue in the run GGG in the lagging (I) and leading (L) orientations, respectively (*19*). Similarly, plasmids designated as pUC-N(I) and pUC-N(L) represent the constructions where AAF is located on the third G residue in the Nar I sequence GGCGCC in the lagging (I) and leading (L) orientation, respectively. (**B**) Plasmids with AAF were constructed with the use of the gapped duplex strategy (*4, 5*). For example, equimolar amounts of pUC-3G(I) linearized with Sca I and pUC-H(I) linearized with Hinc II were mixed, heat-denatured, and allowed to reanneal. Plasmids designated as pUC-H(I) and pUC-H(L) (for pUC-Helper) served in the construction of both of the gapped duplexes in the run of G and the Nar I contexts. They contain a unique Hinc II site where the monomodified oligonucleotide (*20*) was inserted. All the gapped duplexes were similarly formed by mixing of the appropriate parent plasmids. Subsequently, covalently closed plasmids were formed by hybridization of AAF-monomodified oligomers into the corresponding gapped duplexes followed by ligation with T4 DNA ligase. Covalently closed plasmids were purified on CsCl gradients.

Table 1. Mutation frequencies (expressed as percentage) induced by a single AAF adduct on the different plasmid constructions in strain JM103*uvrA6* and w7118*uvrA6,mutS*. The specificity of the two mutation assays was checked by sequencing of randomly picked mutant colonies (blue) derived from all the different constructions. In all cases, the mutations were those expected: GGG → GG and GGCGCC → GGCC for the run of G and the Nar I context, respectively. Plasmids with a single AAF adduct were introduced into bacteria by electroporation. The mutation frequencies were measured under both SOS uninduced (−SOS) and SOS-induced (+SOS) conditions. The SOS system was induced by ultraviolet irradiation at 10 J/m^2 before the transformation step (*4, 5*). Transformed cells were plated on LB agar containing ampicillin (100 mg/liter), 5-bromo-4-chloro-3-indolyl β-D-pyranoside (X-Gal, 50 mg/liter), and isopropyl β-D-thiogalactopyranoside (IPTG, 60 mg/liter). Mutation frequencies were calculated from the ratio of blue to total colonies. Each mutation frequency was measured independently at least three times. The agreement between these determinations was in all cases within a factor of 2. The total number of blue and total colonies that were scored is given in parentheses. Asterisk denotes position of AAF adduct.

Mutagenized sequence	Orientation	JM103*uvrA6*		W7118-*uvrA6,mutS*
		−SOS	+SOS	−SOS
--GGCG*CC--	Leading pUC-N(L)	0.1 (10/9810)	0.51 (102/20120)	0.034 (28/81432)
	Lagging pUC-N(I)	2.23 (137/6140)	10.4 (2790/26940)	0.86 (430/49984)
---GGG*---	Leading pUC-3G(L)	0.001 (1/96298)	0.08 (18/22228)	0.001 (1/100000)
	Lagging pUC-3G(I)	0.037 (52/138792)	1.8 (310/17148)	0.011 (11/100000)

been mapped precisely, it was shown that under normal growth conditions, the switch occurs between the origin and nucleotide 245 (*15*). In our constructions, the adduct is located at nucleotide position 363 and 432 in the leading and lagging strand constructions, respectively. Therefore, replication past the AAF adducts in our constructions is likely to be Pol III–mediated.

Alternatively, mutations may be generated at similar rates in both strands, but a repair mechanism may act preferentially on the leading strand after replication. However, we found that the bias in mutation frequency between the two strands was not affected when the long patch mismatch repair (LPMR) pathway was inactivated (Table 1, *mutS* allele) (*16*). Although this result shows that LPMR does not cause the bias, it does not rule out the possibility that an unknown postreplication correction mechanism contributes to the bias.

No difference in fidelity between leading and lagging strand synthesis was found for spontaneous base substitution errors and deletions in a human HeLa cell extract in vitro: Exonucleolitic proofreading occurred with the same efficiency in both strands (*17*). On the other hand, the lagging strand in *E. coli* is more susceptible than the leading strand to replication-dependent deletions triggered by palindromes (*18*).

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20. The monomodified oligonucleotides (5′-ATCACCGGCG^{AAF}CCACA and 5′-ATACCCGGG^{AAF}ACATC) were used for the constructions at the Nar I and GGG run context, respectively. The monomodified oligonucleotides were purified by high-performance liquid chromatography and characterized as previously described (*4*, *5*).
21. We thank J. Lindsley for comments on the manuscript, I. B. Lambert and R. L. Napolitano for helpful discussions, and I. Pinet for technical assistance. Supported in part by a grant from the Human Frontier Science Program and from the Association pour la Recherche sur le Cancer.

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Signal Sequence Trap: A Cloning Strategy for Secreted Proteins and Type I Membrane Proteins

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A method was developed to clone, without the use of specific functional assays, complementary DNAs (cDNAs) that carry specific amino-terminal signal sequences, such as those encoding intercellular signal-transducing molecules and receptors. The vector used in this system directed the cell surface expression of interleukin-2 receptor fusion proteins when inserts with signal sequences were cloned in-frame with the correct orientation. An expression cDNA library was constructed from a bone marrow stromal cell line, which contained 5′ portion–enriched cDNAs (the average size was 400 base pairs). Two cDNAs that encoded putative cytokine molecules, stromal cell–derived factor–1α (SDF-1α) and SDF-1β, which belong to the intercrine–macrophage inflammatory protein superfamily, were cloned.

Complex interactions between stromal cells and hematopoietic stem (or progenitor) cells are required for hematopoiesis, during which many unknown molecules should be involved in intercellular signal transduction. However, it is difficult and tedious to clone either cDNAs for many different growth factors or adhesion molecules required for transducing specific intercellular signals by establishing a bioassay system specific to each molecule and then measuring the biological activities of unknown molecules. To overcome this problem, we developed a method for trapping signal sequences as a strategy for cloning cDNA for growth factors and type I integral membrane proteins, taking advantage of the specific NH_2-terminal signal sequences that most precursors for secreted factors and transmembrane molecules carry and that are within 400 base pairs (bp) of the 5′ termini of the mRNA (*1*).

The secretory signal sequence leads the NH_2-terminus of the de novo synthesized protein into the endoplasmic reticulum or outside the cell. We constructed the pcDL-SRα-Tac(3′) vector that could direct the cell surface expression of Tac (α chain of the human interleukin-2 receptor) fusion proteins when inserts with signal sequences were cloned in-frame with the correct orientation (*2*). The fusion protein expressed on plasma membranes was easily detected by antibodies to Tac (anti-Tac) (*3*). The pcDL-SRα-Tac(3′) vector had cloning sites between the SRα promoter, a fusion promoter composed of the simian virus 40 early promoter and the R-U5 segment of human T cell leukemia virus type I, and the coding sequence (without a signal sequence) of Tac cDNA (*4*) (Fig. 1A). Using a fragment containing a signal sequence of human granulocyte colony-stimulating factor (hG-CSF) (*5*) as a positive control [hG-CSF(5′)] and a 5′ fragment of the human retinoic acid receptor α (hRAR) (*6*) as a negative control [hRAR(5′)] (*7*), we tested whether the pcDL-SRα-Tac(3′) vector system allowed the transient cell surface expression of the Tac epitope on the transfected cells (Fig. 1B). The Tac antigen was detected by anti-Tac on the surface of COS-7 cells transfected with the hG-CSF(5′)–containing plasmid, which indicates that the fusion protein consisting of hG-CSF(5′) and Tac(3′) was expressed on the cell surface. In contrast, when the hRAR(5′)-containing plasmid was used, the Tac antigen was not detected on the cell surface. When the sensitivity of the detection was tested by serial dilution experiments of the hG-CSF(5′)–containing plasmid with the hRAR(5′)-containing plasmid, one positive clone in a pool of 100 negative clones was easily detected.

We then constructed an expression cDNA library containing 5′ portion–en-

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